REMARKS

Claims 1, 2, 4, 5, 50 and 51 are pending. Claim 1 has been amended. Support for the amendments can be found throughout the application as originally filed. No new matter has been added.

Rejection of Claim 1 Under 35 U.S.C. §112, second paragraph

Claim 1 is rejected under 35 U.S.C. §112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention."

According to the Examiner, claim 1 is indefinite in the "recitation of X_5". Claim 1 has been amended to replace "X_5" with "Xaa_5", thereby obviating this rejection.

In addition, the Examiner asserts "the claim is also indefinite in that 'Z_2' is not defined." Claim 1 has been amended to recite that Z_2 is a polypeptide of at least one amino acid, thereby obviating this rejection.

Rejection of Claims 1, 2, 4, 5, 50 and 51 Under 35 U.S.C. §112, first paragraph

Claims 1, 2, 4, 5, 50 and 51 are rejected under 35 U.S.C. §112, first paragraph, for enablement and written description. In particular, the Examiner states

> there has been no justification for the general formula of claim 1 in the instant specification. The specification states at page 44 that "an amino acid was regarded as preferred at a given position in the sequence if it occurred in five or more isolates." It is not seen that Applicants have justification for arbitrarily picking 5 occurrences to make a general formula ... If applicants are to put forward a general rule they should have some justification for it other than arbitrarily picked numbers of 5 occurrences using the specific conditions.

Applicants respectfully traverse this rejection. Contrary to the Examiner's assertions, the selection of an amino acid as preferred at a particular position based upon its occurrence at that position in 5 or more of the isolates was not random. As provided at pages 21 to 22 of the

application, the complete random library to be scanned for enterokinase cleavage activity was designed to allow for any amino acid except for cysteine to occur at each position within the variegated portion of the display polypeptide. Thus, in such a random library, the likelihood of a particular amino acid being present at a particular position within the variegated region is 1 in 19. That calculates to a 5.2% chance that a particular amino acid will be present at a position in the variegated region of a randomly generated polypeptide display library. Using an enterokinase screening assay, Applicants obtained 90 isolates. The sequence of the variegated region of all 90 of these isolates were determined. If an amino acid was present in 4 or less of the 90 isolates, this indicates that it could be a random occurrence of the amino acid at that position. However, if an amino acid was present at a particular position in 5 or more of the 90 isolates, this is greater than a 5.2% chance of the amino acid occurring at that position randomly. Therefore, the presence of an amino acid at a particular position in 5 or more of the isolates indicates that this is more than a random occurrence of the amino acid at that position, thereby indicating that such an amino acid may play a role in cleavage by an enterokinase. For these reasons, it is clear that the formula set forth in claim 1 is not arbitrary. Therefore, Applicants respectfully request that the Examiner withdraw this rejection.

The Examiner also states that

> [a]n analysis of Tables 1-8 only shows one embodiment of the protein of claim 2, wherein Xaa_5 is Ser. No examples of claim 2 wherein Xaa_5 is Met, Thr, Ala, Asp, Leu, Phe, Asn, Trp, Ile, Gln, Glu, His, Val, Gly or Tyr is found, and this does not even account for the broader embodiment of claim 1.

Applicants respectfully traverse this rejection. Contrary to the Examiner's assertions, Applicants have tested various amino acids at the Xaa_5 position and have demonstrated that all of the amino acids recited for the Xaa_5 position in claim 2 result in enterokinase cleavage. For example, at page 43 of the application, Applicants perform experiments to identify residues that can be on the C-terminal side of the scissile bond, i.e., amino acids that can be at position Xaa_5 of the recited formula. As provided at page 43, lines 27-28, "DNA sequencing of the phage isolates identified phage clones having 16 of the 20 amino acids at the P_1' position following the

Asp-Arg (DR) motif." At page 44, Applicants further tested for enterokinase cleavage when each of these 16 amino acids was present at the Xaa_5 position. Applicants conclude that phage displaying any of Met, Thr, Ser, Ala, Asp, Leu, Phe, Asn, Trp, Ile, Gln, Glu, His, Val, Gly and Tyr were cleaved at certain enterokinase concentrations. See, e.g., page 44, lines 17-22 of the application. Thus, Applicants have clearly demonstrated that Xaa_5 can be any of the amino acids recited in claim 2.

In addition, the application further provides support that Xaa_5 can be any amino acid as recited in claim 1. As provided above, Applicants have demonstrated that 16 of the 20 amino acids can be present at position Xaa_5 and result in a cleavable enterokinase site. In addition, at pages 43 to 44, Applicants provide that "only four amino acids were not observed in any of the isolates at the P_1' position following Asp-Arg, among those isolates sequenced: Lys, Pro, Arg and Cys (which was not permitted in the 13-mer variable portion when the substrate phage library was generated). Applicants go on to state that "the absence of any phage isolates exhibiting these amino acids at the P_1' position does not mean that an EK recognition sequence ... having Lys, Pro, Arg or Cys at the Xaa position will not be cleaved; rather it indicates that such recognition sequences will be cleaved less efficiently than recognition sequence having other amino acids at the Xaa (P_1') position." Thus, it is clear that the Xaa_5 position of the formula in claim 1 can be any amino acid.

For the reasons discussed above, Applicants respectfully request that the Examiner withdraw this rejection.

The Examiner further asserts

> The specification teaches ... that "[t]he exogenous polypeptide was an 86-mer fusion protein having tandem ligand recognition sequences, a variegated segment of thirteen amino acids serving as a template for potential EK recognition sequences, a factor Xa cleavage site, segments linking the foregoing domains and linking the N-terminus of gene III protein" and ... that the tandem ligand recognition sites are "a linear binding sequence, [and] a constrained streptavidin binding loop. The instant claims are now limited to a "ligand recognition sequence", not tandem ones, with no limitation as to a Xa cleavage site or gene III. The potential EK recognition sequence is 7 amino acids not 13.

It is maintained that absent convincing proof to the contrary, these additional limitations in the example have some effect upon which sites will be cleaved by enterokinase and are important in defining an enterokinase cleavage protein.

Applicants respectfully traverse this rejection. The Examiner requests evidence that in the absence tandem ligand recognition sequences, Xa cleavage sites or gene III, the claimed fusion peptides will be cleaved by enterokinase. The Examiner also appears to object to the structure recited in the claims being a 7-mer versus a 13-mer. However, the present application already provides evidence that the recited structure will be cleaved without the presence of these additional elements. Specifically, at page 40 of the present application, Applicants prepared several synthetic peptides that had no ligand recognition sequence (Z_1), had the recited EK cleavage site of Xaa_1-Xaa_2-Xaa_3-Xaa_4-Asp-Arg, and had three amino acids making up the Xaa_5-Z_2 portion of the fusion protein. Applicants demonstrated that all of these synthetic peptides were completely cleaved by enterokinase between the Arg and Xaa_5. Thus, it is clear that without the additional elements recited by the Examiner, the claimed fusion peptides are cleaved by enterokinase.

Applicants also have additional data demonstrating that a fusion protein having an enterokinase cleavage site but none of the other structures required by the Examiner was repeatably cleaved in the presence of low levels of enterokinase. The fusion protein used in these experiments included a prosequence, a Kex2 cleavage site, a 6-mer enterokinase cleavage site (DINDDT) and a kunitz domain. Applicants demonstrated that by using the recited enterokinase cleavage site a kunitz protein could be repeatable cleaved from the fusion protein without any impurities. Thus, it is clear that the enterokinase cleavage site as recited in the claims is sufficient to result in the cleavage of a fusion protein without the presence of a tandem ligand recognition sequence, a Xa cleavage site or a gene III sequence. In addition, it is clear that the 6-mer structure recited in the claims is sufficient for enterokinase cleavage.

For the reasons discussed above, Applicants respectfully request that the Examiner withdraw this rejection.

Enclosed is a check for the Petition for Extension of Time fee. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

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